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[Ca²⁺]_i in Human Heart Failure: A Review and Discussion of Current Areas of Controversy

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Multiple abnormalities have been reported in the setting of human heart failure. It is unclear whether detected changes reflect adaptive alterations in myocardium subjected to increased and sustained hemodynamic overload or are pathogenic to the disease process. As a result of the observation that the primary defect in heart failure is decreased pump function, investigators have concentrated their efforts on determining systolic [Ca²⁺]_i as a logical corollary and a causative mechanism for contractile dysfunction. A simple cause and effect relationship has therefore been proposed with regard to contractile dysfunction and [Ca²⁺]_i. Yet some investigators have found no difference in peak systolic [Ca²⁺]_i between failing and non-failing human myocardium, whereas others have found peak [Ca²⁺]_i to be significantly reduced in failing hearts. Resting calcium concentrations have been reported either to be elevated in failing human myocardium or not different from non-failing human myocardium. Investigators should now appreciate that the force-calcium relationship is not a simple relationship. One must take into account the prolonged time course and slowed mobilization of [Ca²⁺]_i as opposed to simply peak [Ca²⁺]_i. When put in perspective of mechanisms and determinants of the Ca²⁺-force relationship, we begin to realize that failing human myocardium has the "potential" to generate normal levels of force. Only when stressed by [Ca²⁺]_i overload and/or frequency perturbation does myocardium from patients with end-stage heart disease demonstrate contractile failure. Although [Ca²⁺]_i availability and mobilization are likely to play a role in the systolic as well as diastolic dysfunction reported in human heart failure, it is likely that other mechanisms are involved as well (e.g., myocardial energetics). Myocardial energetics is directly related to [Ca²⁺]_i and mobilization in failing human myocardium, because metabolites, e.g., ADP, inhibit pumps, such as sarcoplasmic reticulum Ca²⁺ ATPase activity. We therefore conclude that there is a role for intracellular calcium mobilization and myocardial energetics for systolic and diastolic dysfunction seen in human heart failure.

With the development of cardiac transplantation programs, human myocardium has become available for study. However, studies utilizing human myocardium harvested at the time of cardiac transplantation have only afforded an ice-pick view of an end-stage process. Furthermore, studies are flawed because the etiologies often differ or are unknown, the duration of heart failure varies between patients and there are differing pharmacologic therapies being received by individual patients. Most concerning is that there are no true "normal" hearts available for study and comparison. Despite these limitations,

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^bAbbreviations: DPBA, 12-deoxyphorbol-13-isobutyrate-20-acetate; I_{Ca}, calcium channel current density; SR, sarcoplasmic reticulum; DCM, dilated cardiomyopathy.

studies using human myocardium have yielded important new insights into cellular mechanisms of the end-stage process.

There is a relationship between the force of myocardial contraction and calcium. A simple cause and effect relationship has, therefore, been proposed with regard to contractile dysfunction and $[Ca^{2+}]_i$ in failing human hearts (Figure 1). Other proposed mechanisms involved in contractile failure include: 1) down-regulation of β -receptors; 2) decreased myofilament calcium responsiveness; 3) decreased myofibrillar Mg ATPase

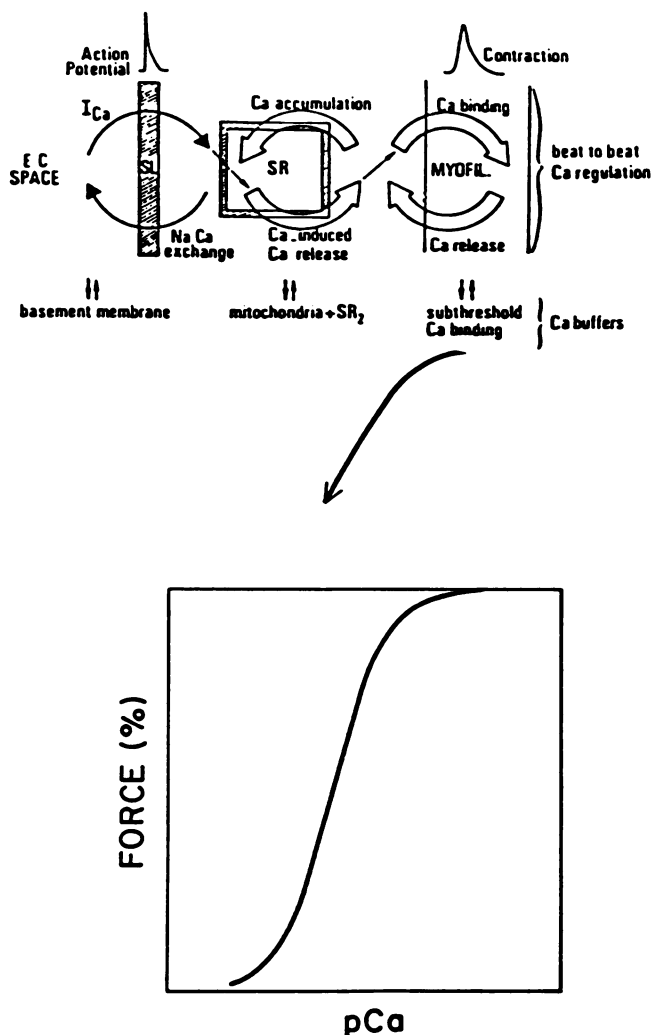


Figure 1. Schematic of calcium movements within in cardiocyte. When the sarcolemma is depolarized, a small amount of Ca^{2+} enters through voltage-dependent calcium channels. This small amount of calcium induces the release of a much larger amount of Ca^{2+} from the sarcoplasmic reticulum (Ca^{2+} -induced calcium release) that then binds to the myofilaments resulting in contractile activation. Relaxation occurs when the calcium is resealed into the SR and removed from the cytosol via the Na-Ca exchange and sarcolemmal Ca^{2+} ATPase. Mitochondria serve as calcium stores and buffers. Ca = calcium, EC = extracellular space, SR = sarcoplasmic reticulum. I_{Ca} = transsarcolemmal calcium current. By performing skinned fiber experiments where the sarcolemma, SR and mitochondria are destroyed, force can be plotted vs. known bath calcium concentrations ($pCa = -\log [Ca^{2+}]$) and myofilament calcium responsiveness directly studied. The force- $[Ca^{2+}]$ relationship is fit to the modified Hill relationship.

activity and decreased myofibrillar protein content; and/or 4) decreased energy supply. Experimental studies on non-failing and diseased human hearts have supported some of these hypotheses and rejected others. The mechanism of contractile failure seen with heart failure, therefore, remains elusive.

β -RECEPTOR FUNCTION

It has been clearly demonstrated that β_1 receptors are down-regulated while β_2 receptor number is unchanged in the setting of heart failure [1]. As demonstrated in Figure 2, inotropic response to increasing concentrations of isoproterenol, a non-selective β -receptor agonist, is diminished in right ventricular trabeculae carneae isolated from failing human hearts (open triangles) compared to muscles from non-failing hearts (open circles). However, addition of forskolin, which directly activates adenylate cyclase coupling β -receptors via G proteins to intracellular ATP hydrolysis, produces similar contractile responses in muscles from failing and non-failing human hearts. These data suggest that adenylate cyclase activity is intact in failing human hearts.

It has been accepted that the changes in β -receptor number and function is not pathogenic in the disease process and more likely reflect adaptive responses of the failing heart to excess catecholamine stimulation [1]. This premise is supported by studies with β -receptor antagonists, which, despite dramatic improvement in cardiac function, result in only partial recovery of β -receptor numbers [2]. Furthermore, in a study using carvedilol, which does not affect β -receptor number, there was a significant improvement in cardiac function despite an unchanged receptor number [3]. This would appear to uncouple the number of β -receptors as being causally related to contractile failure.

MYOFILAMENT CALCIUM SENSITIVITY AND ATPase ACTIVITY

A decrease in maximal force or myofilament calcium responsiveness (i.e., a change in cooperativity or slope of the force calcium relationship or calcium concentration

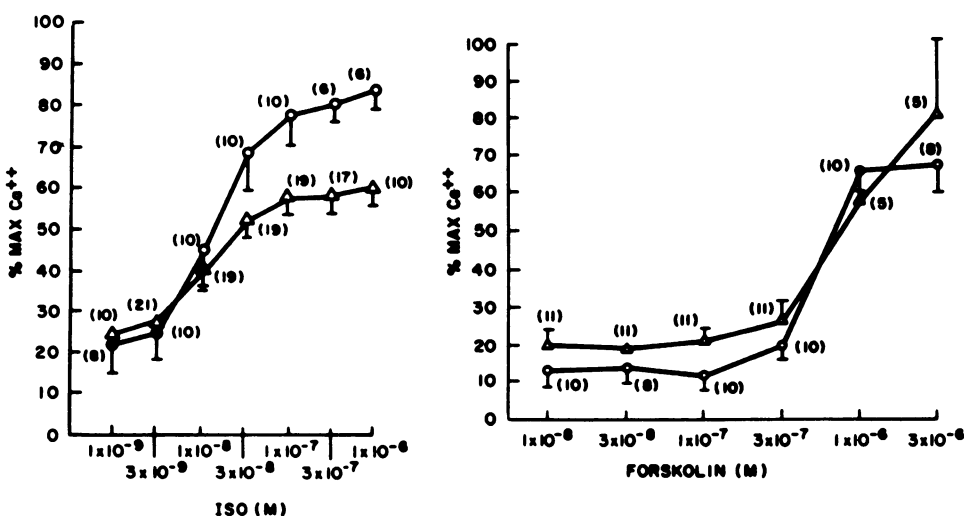


Figure 2. Isolated trabeculae carneae from non-failing and failing human hearts were stretched to the peak of the active tension curve and electrically stimulated to contract. Response to increasing concentrations of isoproterenol and forskolin on isometric twitch force expressed as a percent of maximal twitch response to high $[Ca^{2+}]_o$. ISO = isoproterenol, M = moles/liter; percent MAX Ca^{++} = twitch force expressed as a percent of maximal response to high calcium concentration the bath. Δ = myopathic human muscles. o = control human muscles.

required for 50 percent contractile activation ($[Ca^{2+}]_{50\%}$) has been proposed as explanation for the decreased contractile performance of failing human hearts. Maximal force developed by chemically skinned trabeculae carneae of patients with heart failure has been shown to be similar to that of non-failing myocardium [1, 5]. Figure 3 demonstrates maximal calcium-activated force, Hill coefficients and calcium required for 50 percent contractile activation in non-failing and myopathic fibers using two techniques, i.e., steady-state tetani in intact muscle preparations and saponin skinned fibers. Figure 4 demonstrates a non-signal averaged force and calcium trace of a twitch and steady-state activation. The Ca^{2+} sensitivity of the myofilaments in chemically skinned [4-6] and intact fibers [4] from failing human hearts are comparable to that of non-failing myocardium.

As demonstrated in Figure 5, increasing peak $[Ca^{2+}]_i$ results in contractile activation that is similar for failing (open triangles) and non-failing human myocardium (open circles) when agents like acetylthiocholine, a digitalis-like compound, and DPI 201-206, a sodium channel agonist and calcium sensitizer, are applied. Both agents increase intracellular calcium via sodium-calcium exchange. These experiments support the idea that changes in myofilament calcium responsiveness do not play a direct role in contractile failure. Furthermore, myofilament responsiveness to changes in $[Ca^{2+}]_i$ is similar for failing and non-failing human myocardium.

Despite similar force-pCa relationships, there are, nevertheless, changes at the level of the thin myofilaments. Hajjar et al. first demonstrated that changes at the level of the thin myofilaments may at times only be detected when agents targeted at the level of the thin myofilaments are applied [5]. Addition of 1 μM DPI 201-106 resulted in a 0.2 pCa unit shift in the force-pCa relationship in myopathic human myocardium with no change in fibers from non-failing hearts [5]. It is interesting that myopathic human myocardium tended to have a slightly higher Hill coefficient that tended to increase in the presence of DPI 201-106 (not significant). Addition of 12-deoxyphorbol-13-isobutyrate-20-acetate (DPBA)^b, which simulates protein kinase C activity, affected the Hill parameters differently in non-failing and failing human myocardium [7]. The $[Ca^{2+}]_i$ required for 50 percent

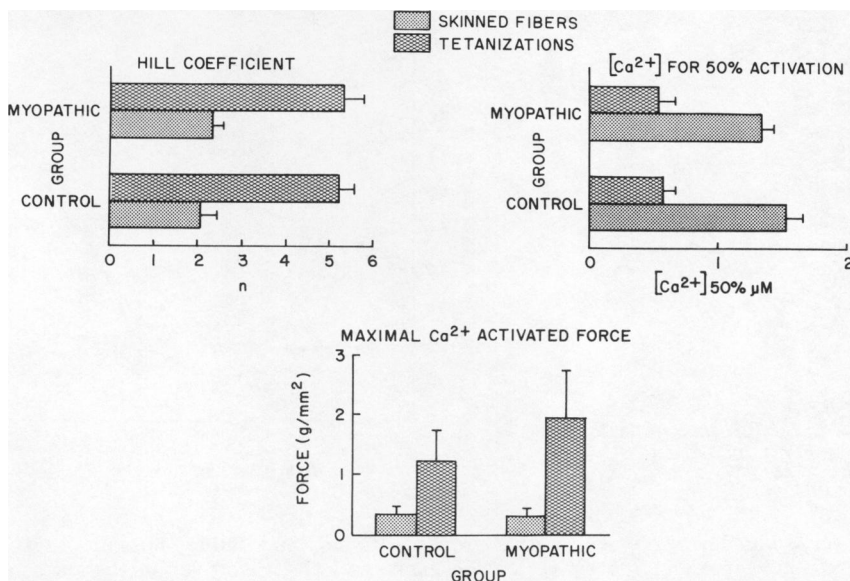


Figure 3. Intact and skinned muscle fibers from non-failing and failing human hearts. Intact muscles were tetanized by stimulating at 50 Hz with a long pulse duration. Fibers from the same hearts were chemically skinned with saponin. Maximal calcium-activated force was measured at pCa = 4; g/mm² = grams/millimeter²; μM = micromoles of calcium.

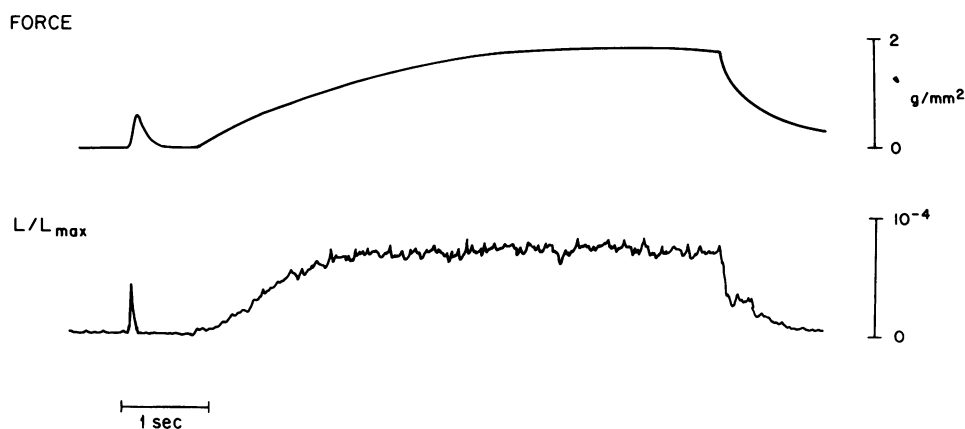


Figure 4. Non-signal averaged force and calcium trace of a twitch and steady-state activation in human trabeculae. g/mm^2 = grams/millimeter²; L/L_{max} = $-\log$ nanoamperes/ $-\log$ maximal nanoamperes from the photomultiplier tube (see Ref. 14 for details).

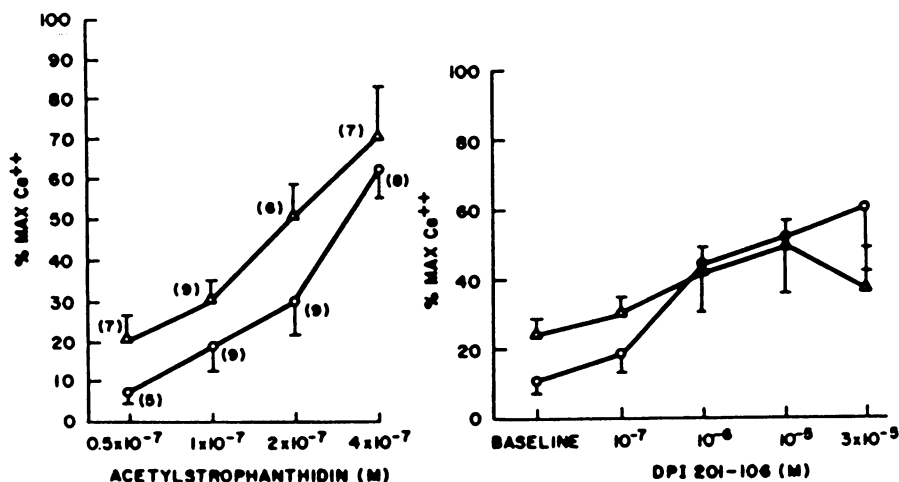


Figure 5. Isolated trabeculae carnae from non-failing and failing human hearts were stretched to the peak of the active tension curve and electrically stimulated to contract. Response to increasing concentrations of acetylthioanthidin and DPI 201-106 on isometric twitch force expressed as a percent of maximal twitch response to high $[Ca^{2+}]_o$; M = moles/liter; percent MAX Ca^{2+} = twitch force expressed as a percent of maximal response to high calcium concentration in the bath; Δ = myopathic human muscles; o = control human muscles.

activation of the myofilaments in steady-state force-calcium relationships obtained with tetani was decreased by 0.09 pCa units in non-failing myocardium vs. 0.21 pCa units for failing myocardium. The Hill coefficients for steady-state contractions were decreased by 2.4 units in non-failing and increased by 0.4 units in failing myocardium. For twitch force, the $[Ca^{2+}]_{50\%}$ was increased 0.11 and 0.13 pCa units for non-failing and myopathic myocardium, respectively. Interestingly, the Hill coefficient for twitch was decreased by two units in nonfailing human myocardium, as opposed to only a 0.81 unit decrease in myopathic myocardium [7]. These data indicate that not only are there differences at the level of the thin myofilaments but that these differences may be reflected in changes in myofilament calcium responsiveness if challenged with agents targeted to act at the level of the thin myofilaments.

A 25 percent decrease in myofibrillar ATPase activity has been reported in failing hearts along with a 20 percent decrease in myofibrillar protein content in ischemic and idiopathic dilated cardiomyopathy by two separate groups, as well as our laboratory (Liao, unpublished data) [8, 9]. If in the presence of an elevated resting intracellular calcium concentration there was normal myofibrillar ATPase activity, the force-calcium relationship would be shifted upward in the diastolic ranges, thereby reducing contractile reserve [12]. A lower MgATPase activity might, therefore, prove beneficial in the setting of heart failure where slower cross-bridge cycling rates have been reported as well as diminished substrate availability for energy supply [10, 11].

Dynamic stiffness spectral analysis was performed on trabeculae carneae obtained from non-failing hearts of brain-dead organ donors ($n = 5$), hearts of patients undergoing surgery for mitral stenosis ($n = 4$) and patients with end-stage heart failure from dilated cardiomyopathy ($n = 6$). The left ventricle from patients with mitral stenosis was assumed normal because it is relatively protected, and patients demonstrated no change in left ventricular function. As the length was oscillated sinusoidally, the force outputs were sinusoidal in all three groups. At low frequencies of oscillations, the amplitude of force oscillation was constant in both non-failing and failing muscles. As the frequency increased, the amplitude of force decreased and reached a minimum at about 0.78 ± 0.06 Hz ($n = 5$) for non-failing muscles, 0.51 ± 0.09 ($n = 4$) for the mitral stenosis group ($p = .036$ compared to controls) and 0.42 ± 0.06 Hz ($n = 6$) for myopathic muscles ($p = .002$, compared to controls). Cross-bridge cycling rates for muscles from failing hearts and hearts with mitral stenosis were not different ($p = .4$). Further increases in the frequency of oscillation resulted in a similar increase in stiffness for all experimental groups, which reached a plateau above 30 Hz. This clearly shows that 1) the cross-bridge cycling rate in myopathic muscles is reduced and 2) the number of force-generating cross-bridges is the same in non-failing and myopathic hearts under ideal conditions of substrate and maximal calcium-activation. These findings also indicate that care should be exercised when comparing samples from presumed "normal" or "control" hearts (i.e., mitral stenosis or patients with coronary artery disease).

PEAK CYTOSOLIC $[Ca^{2+}]_i$, CALCIUM MOBILIZATION AND CONTRACTILE ACTIVATION

As a result of the long-held belief that the major defect in heart failure is decreased contractility, the pharmaceutical industry has focussed on the development of agents that increase $[Ca^{2+}]_i$ and result in positive inotropy. However, the use of positive inotropes has not proven to be a panacea. This has resulted in the formulation of the second corollary that heart failure is an end-stage process that is not amenable to pharmacologic intervention with regard to progression or reversal of the disease process. One of the first abnormalities detected in cardiac fibers from patients with end-stage heart failure was an abnormal intracellular $[Ca^{2+}]_i$ handling first reported by Gwathmey et al. [13]. The abnormality in myopathic muscles loaded with the bioluminescent photoprotein aequorin was characterized by a prolonged $[Ca^{2+}]_i$ transient with an additional $[Ca^{2+}]_i$ signal (L_2). They also found that diastolic $[Ca^{2+}]_i$ was elevated in myopathic fibers whereas systolic $[Ca^{2+}]_i$ was unchanged at a time when there was an often blunted augmentation of peak contractile force in failing myocardium compared to non-failing myocardium [14]. In contrast, $[Ca^{2+}]_i$ transients recorded from isolated single cells from failing hearts loaded with the Ca^{2+} indicator fura-2 were found to have decreased systolic peak $[Ca^{2+}]_i$, but elevated diastolic $[Ca^{2+}]_i$ [15]. However, Lederer et al. found that in isolated myocytes loaded with the calcium indicator Indo peak $[Ca^{2+}]_i$ attained during a 60 mV depolarization was ~ 1 μM , similar to 1.2 μM seen in multicellular preparations stimulated at 0.33 Hz loaded

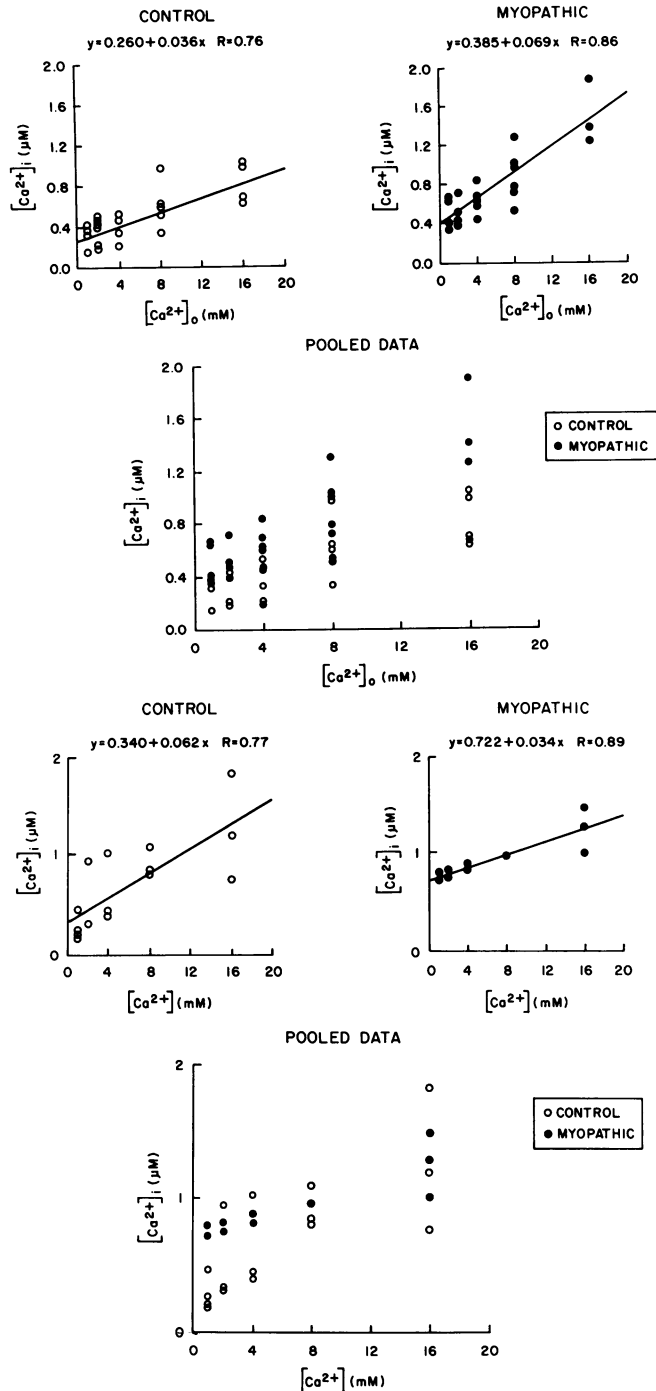


Figure 6A (top three panels). Aequorin determined peak $[Ca^{2+}]_i$ in micromoles (μ M) for twitch in control and myopathic human myocardium in the presence of increasing $[Ca^{2+}]_o$ in millimolar (mM). Non-failing myocardium = open circles; failing myocardium = closed circles. **Figure 6B (bottom three panels).** Peak $[Ca^{2+}]_i$ for steady-state tetanizations in the presence of increasing $[Ca^{2+}]_o$ in the absence of ryanodine.

with aequorin [22]. Further, Lederer et al. [22] reported higher resting intracellular calcium concentrations with repetitive stimulations (~ 375 nM) similar to our findings in multicellular preparations. They proposed that the increase in contractile force was more dramatic than the increase in the amplitude of the $[Ca^{2+}]_i$ transients that were superimposed on a rising diastolic $[Ca^{2+}]_i$ although there was progressive loading of the sarcoplasmic reticulum. Also, Vahl et al. found that peak $[Ca^{2+}]_i$ in fura-2 loaded trabeculae carneae were similar for isometrically contracting non-failing and failing muscle preparations [16]. With afterload reduction, there were, however, alterations in the shape of the calcium transient: the amplitude increased, the time to peak was delayed and at lower afterloads, a long-lasting plateau was observed and the diastolic decay was slowed [16]. Demonstrated in Figure 6A is aequorin-determined peak $[Ca^{2+}]_i$ for twitch in non-failing (open circles) and failing human myocardium (closed circles) in the presence of increasing $[Ca^{2+}]_o$. Pooling the data revealed no difference in peak $[Ca^{2+}]_i$ for twitch. Also shown in Figure 6B is peak $[Ca^{2+}]_i$ for steady-state tetanizations in the presence of increasing $[Ca^{2+}]_o$. Again, peak $[Ca^{2+}]_i$ were similar for non-failing (open circles) and failing muscles (closed circles). These data would suggest that failing human myocardium has sufficient $[Ca^{2+}]_i$. We propose that failing myocardium should benefit from improvement in calcium mobilization.

An important observation is that at lower stimulation rates and at 1 Hz at 35-37°C, there is no difference in peak contractile performance in failing and non-failing human myocardium. Figure 7 shows the effect of increasing the temperature from 30°C to 37°C on the calcium transient. This experiment demonstrates that the occurrence of the two components in the calcium transient is not an artifact of the lower temperature at which earlier aequorin experiments in failing human myocardium were performed [13]. These data are further supported by a recent report in muscles from patients with hypertrophic

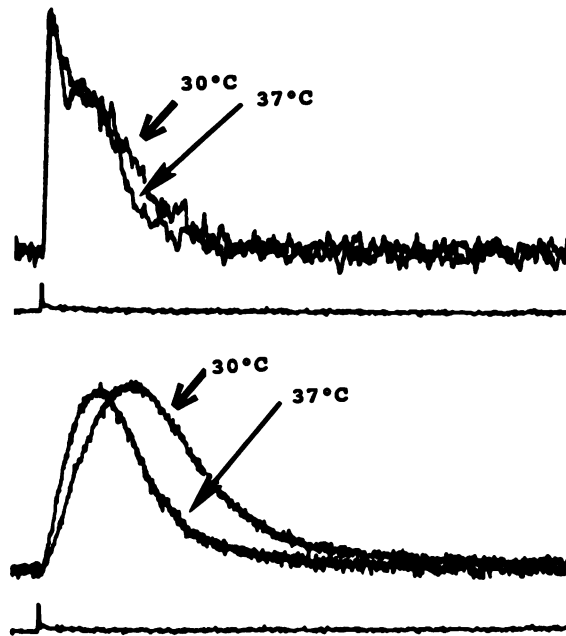


Figure 7. Shows the effect of increasing the temperature from 30°C to 37°C in a muscle demonstrating two components to the calcium transient. Upper trace denotes calcium transient. Arrow denotes transient at 37°C. Note the abbreviation in time course. The lower panel demonstrates the effect of increasing temperature (37°C) on isometric twitch force. Again note the abbreviation at the higher temperature (arrow).

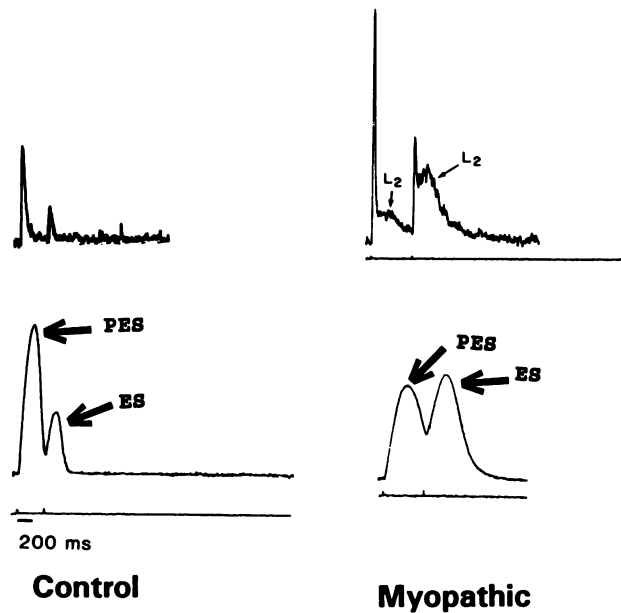


Figure 8. Upper panels demonstrate calcium transients as detected with aequorin. Lower panels demonstrate associated contractile force in isometrically contracting trabeculae carneae. Using paired electrical stimulation in the case of control myocardium, there is an associated decrease in the amplitude of both the calcium transient and force for the extrasystole (ES). The post-extrasystole (PES) is associated with greater contractile force and a larger calcium transient. In myopathic myocardium, a greater amplitude of the force of contraction was observed for the extrasystole (ES). The amplitude of the first fast component of the $[Ca^{2+}]_i$ signal is greatly diminished compared to the PES. There is a large increase in the amplitude of the second slower calcium transient component (L_2).

cardiomyopathy studied at 37°C [17]. The observation of similar peak twitch force at slower stimulation rates has been confirmed in numerous laboratories [18-21].

A potential greater dependency of contractile activation on the amplitude of the second slower component as opposed to the fast first component is suggested in Figure 8. Using paired electrical stimulation, which is similar to a premature contraction or extrasystole with a postextrasystole, a greater amplitude of the force of contraction was observed both for the post-extrasystole and extrasystole in failing myocardium. The amplitude of the first fast component of the $[Ca^{2+}]_i$ signal is greatly diminished in the case of the extrasystole as opposed to the post-extrasystole, which is associated with greater SR calcium release. The second slower component, however, was significantly larger during the extrasystole. It has been demonstrated that an increase in the amplitude of L_2 results in potentiation of the extrasystole in myopathic muscles and most likely reflects activation of the myofilaments by calcium [14]. In the case of non-failing myocardium, there is an associated decrease in the amplitude of both the calcium transient and force associated with the extrasystole. The post-extrasystole was associated with an increase in SR calcium release and force.

AREAS OF CONTROVERSY

The appearance of two components seen in multicellular preparations has been found by some investigators in isolated myocytes using the calcium indicator Fura-2 [23]. This group reports similar peak SR calcium release concentrations as found in other species including the dog and rat (Ruth Altschuld, personal communication). However, there has

been a report in isolated myocytes using the fluorescent indicator Fura-2 that peak SR calcium release is diminished by about 50 percent [15]. The study with Fura-2 did not report cell shortening nor force. This report, however, found a significant increase in resting calcium concentration similar to earlier reports (172 percent higher compared to 148 percent higher in multicellular preparations using aequorin) [14]. As a result of the aforementioned data, the current debate would appear to be reduced to intracellular calcium ability because of the known relationship between calcium and force. There are, however, several compelling lines of evidence that suggest that intracellular calcium handling, though in part responsible for contractile performance, cannot be the sole factor involved.

It was recently demonstrated in severely hypertrophied myocardium from patients with hypertrophic cardiomyopathy that agents that increased intracellular calcium concentration further impaired systolic as well as diastolic performance [17]. This is similar to findings in patients with end-stage heart failure due to ischemic cardiomyopathy and idiopathic cardiomyopathy [23, 24]. However, agents that improved SR calcium mobilization function prevented and/or improved the detrimental effect of increased intracellular calcium concentration in the presence of frequency perturbation [17, 23, 24].

How do $[Ca^{2+}]_i$ determinations fit with several other observations regarding calcium channel number and function, and sarcoplasmic reticulum calcium release? Calcium channel number has been reported to be increased in the setting of hypertrophic cardiomyopathy [25], decreased [26] or unchanged in the setting of idiopathic dilated cardiomyopathy and ischemic cardiomyopathy [20, 27]. The reported increase in calcium channel number in hypertrophic cardiomyopathy might explain in part the reported negative inotropic effects of agents that increase transsarcolemmal calcium in flux, i.e., increased extracellular calcium concentration [17]. However, it would not support similar observations in the presence of digitalis, an alkaloid that acts via an increase in intracellular sodium and resultant sodium-calcium exchange. Therefore, a change in calcium channel number may or may not contribute to reported differences in $[Ca^{2+}]_i$. In the case of idiopathic dilated cardiomyopathy, the picture is less clear. There are reports that the calcium channel number is reduced by 20 percent as detected with radioligand binding studies or unchanged [20, 26, 27]. Associated with the observation of a decrease in calcium channel number there was a 20 percent decrease in mRNA for the α_1 subunit of the dihydropyridine receptor subunit (Dr. James D. Marsh, personal communication). These observations would suggest a reduced transsarcolemmal calcium flux with resultant diminished SR calcium loading and/or release (e.g., calcium-induced calcium release). Interestingly, despite reports of a reduced number of calcium channels, calcium channel current density (I_{Ca}) has been demonstrated to be unchanged in myocytes isolated from failing human hearts [28]. In fact, the I_{Ca} tended to be higher (I_{Ca} -4.7 ± 2.7 vs. -3.8 ± 1.2 $\mu A/cm^2$ for failing and non-failing, respectively), which would support physiology experiments. The lack of statistical significance most likely reflects the rather large variance in the myopathic fibers and relatively small number of non-failing myocytes studied. Further, activation and inactivation of I_{Ca} were found to be similar to myocytes isolated from non-failing human myocardium [28].

At faster stimulation rates, myopathic human myocardium demonstrates a progressive abbreviation in action potential duration and, thereby, may affect transsarcolemmal calcium flux via calcium channels as well as SR calcium release [14]. This observation has been supported by a report that peak I_{Ca} decreases as the contractions increase with repetitive stimulation in isolated human myocytes from patients with heart failure [22]. The effect of increasing stimulation rate on force and action potential configuration is demonstrated in Figure 9. Notice that with progressive abbreviation in action potential duration there is an increase in diastolic force and a decrease in peak twitch force in failing

myocardium. The role of changes in calcium channel number and function in relation to the contractile activation seen in the setting of heart failure remains elusive.

With regard to sarcoplasmic reticulum calcium loading and release, the experimental findings have similarly been varied. Movsesian et al. have reported that sarcoplasmic reticulum (SR) sequestration in failing human myocardium is not different from non-failing myocardium [29]. Further, they suggest that phospholamban activity is not altered [30]. In contradistinction to these experimental findings, SR mRNA levels for SR Ca^{2+} ATPase activity have been reported to be decreased [31]. Limas et al. report that SR function is decreased in the setting of failure (3.3 ± 0.6 vs. 6.5 ± 0.5 nmol Ca^{2+} /mg/min, $p < .01$) using a similar preparation as used by Movsesian [32]. It is important to note that Limas et al. also reported significantly reduced calcium uptake by the sarcoplasmic reticulum at 4 to 5 months post-transplantation (4.5 ± 0.5 nmoles Ca^{2+} /mg/min compared to 5.6 ± 0.5 nmoles Ca^{2+} /mg/min, $p < .01$) [33]. Statistical analysis, however, would indicate that the post-transplantation and dilated cardiomyopathy sarcoplasmic reticulum defect is the same ($p = .25$). This then raises the question of whether the reported decreases in sarcoplasmic reticulum Ca^{2+} ATPase activity are causally related to the systolic contractile failure seen with heart failure. The transplanted hearts for a similar reduction in SR calcium uptake activity were not in systolic failure. The observation of reduced activity, however, would support observed impairment of diastolic relaxation seen in transplanted hearts [33]. A 30 percent decrease in SR Ca^{2+} ATPase message (with no change in total number of pumps but a decrease in density) has been reported. It has been suggested that these changes might contribute to the diastolic dysfunction seen in failing human myocardium but would not support the idea of a significantly reduced calcium uptake or release by the SR [34]. Interestingly, using hyperpermeable skinned fiber preparations it has been demonstrated that the calcium sequestering ability of myocardium from patients with end-stage heart failure is similar if not higher (maximum Ca^{2+} uptake at pCa 7.0, 1.81

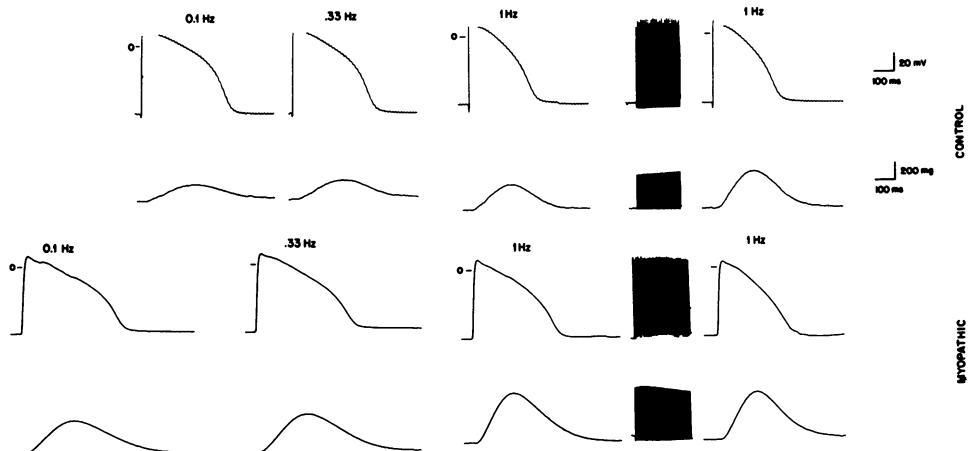


Figure 9. Multicellular action potentials recorded in trabeculae carneae electrically stimulated from non-failing and failing human myocardium. The muscles were stimulated at increasing rates (Hz). Upper panels are action potentials for both control and myopathic myocardium. Lower panels are associated isometric twitches. Experiments were performed at 30°C, pH = 7.4, $[Ca^{2+}]_o = 2.5$ mM. Note the relatively rapid stabilization of action potential duration in the trabeculae from the non-failing heart. This was associated with an increase in twitch force. In contrast, there is progressive abbreviation of action potential duration in the muscle from a failing heart. Instead of increasing twitch force there was a decrease in peak twitch force and an increase in end-diastolic force at the higher stimulation rate.

and 2.68 nmol Ca^{2+} /mg fiber protein in non-failing and failing human myocardium, respectively) than seen in non-failing myocardium [6]. There was no difference in the extent (0.71 ± 0.06 vs. 0.65 ± 0.07 in DCM) and rate of caffeine-induced Ca^{2+} release (0.08 ± 0.03 vs. 0.06 ± 0.01 in dilated cardiomyopathy [DCM]). These observations would support findings of similar SR calcium release [13, 16, 22], but would not support other studies [35, 36].

How can one reconcile differences in experimental findings, and more importantly, do the differences provide important insights into the pathophysiology and/or pathogenesis of heart failure? What do these differences tell us with regard to the study of human heart failure, and is there functional importance associated with these observations? A piece of the puzzle might lie in the observation that changes in the frequency of stimulation alter the action potential duration with resultant graded and fluctuating SR calcium release [14]. In addition, another piece of the puzzle may lie in the observations that depending on the level of depolarization that SR calcium-release can vary in amount and duration [22, 37]. Yet another piece of the puzzle may lie in our recent observations that pharmacologic interventions may alter transmembrane signalling and calcium channel number [38]. We have recently studied a model of idiopathic dilated cardiomyopathy in the turkey poult that demonstrates similar pathophysiology as seen in human heart failure and found calcium channel number to change in a complex manner (e.g., initial increase, followed by a decrease and finally another increase) [39]. Although the changes in calcium channel number were large compared to experimental findings in human myocardium, a functional significance was suggested by the experimental findings that the nifedipine-contraction response relationship was shifted by 0.5 log units higher in hearts that demonstrated an increased number of calcium channels. Similar functional experiments have not been performed in failing human myocardium. Because our study was a longitudinal study without pharmacologic intervention, it would appear that some of the differences reported in human myocardium may reflect differences in stages and/or duration

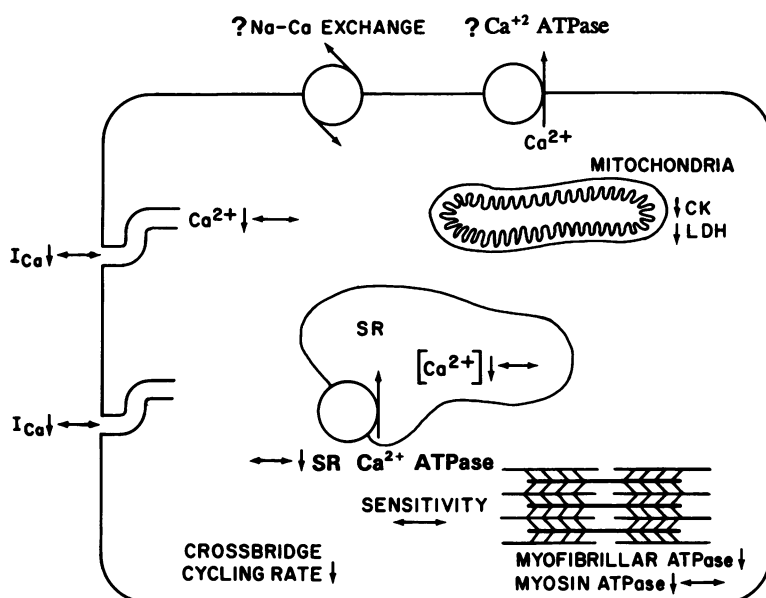


Figure 10. Abnormalities in excitation-contraction coupling in human myopathic hearts. CK = creatine kinase; LDH = lactate dehydrogenase; I_{Ca} = calcium current; SR Ca^{2+} ATPase = sarcoplasmic reticulum calcium ATPase; Ca^{2+} ATPase = sarcolemmal calcium ATPase; ↑ = increase; ↓ = decrease; ↔ = no change.

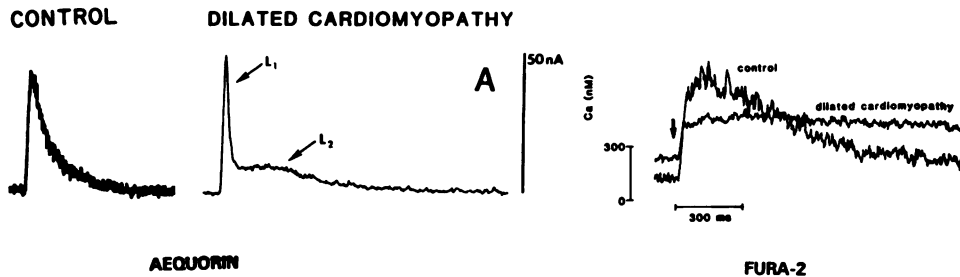


Figure 11. Force and $[Ca^{2+}]_i$ transients recorded from aequorin-loaded trabeculae and fura-2 loaded cells of normal and myopathic hearts (by permission of Dr. Beuckelmann).

of disease (although all hearts are classified as demonstrating heart failure reflected in reduced ejection fractions) and potential effects of pharmacologic interventions.

THE RELATIONSHIP BETWEEN FORCE AND CALCIUM: IMPLICATIONS FOR CONTRACTILE PERFORMANCE

Figure 10 illustrates the abnormalities in excitation-contraction coupling that have been reported so far in myopathic hearts. Since different investigators using different experimental procedures and calcium indicators have obtained contrasting results, intracellular $[Ca^{2+}]_i$ handling in heart failure remains a controversial issue. Gwathmey et al. found that systolic $[Ca^{2+}]_i$ transients in trabeculae carneae from patients with end-stage heart failure were similar to that of control muscles [14]. They also observed an additional calcium signal (L_2) indicating a prolongation of the diastolic $[Ca^{2+}]_i$ [13]. Beuckelmann et al. found that in single cells, diastolic $[Ca^{2+}]_i$ is higher in diseased cells similar to a report by Gwathmey et al. [14, 15]. In contrast to Gwathmey's group, these investigators found that peak $[Ca^{2+}]_i$ was diminished in myopathic cells, leading Beuckelmann et al. [15] to propose that their results may explain the depressed contractility *in vivo* in patients with heart failure. This conclusion, however, implicitly implies that peak $[Ca^{2+}]_i$ during a twitch determines force development. However, only during steady-state activation, such as in tetanizations or activations in skinned fibers, can the force- $[Ca^{2+}]_i$ relationship truly reflect myofilament responsiveness to Ca^{2+} [40].

Beuckelmann et al. [15] have demonstrated calcium transients with extremely long time courses. In fact, these transients appear to plateau in a similar way to the second slower component reported in aequorin loaded preparations and, therefore, might be likened to steady-state tetanizations. Figure 11 shows $[Ca^{2+}]_i$ transients recorded from aequorin-loaded trabeculae and fura-2 loaded cells of non-failing and myopathic hearts. Examining these transients by time-averaging the $[Ca^{2+}]_i$ transient, $1/\tau \int Ca(t).dt$ where τ represents the duration of the transient, we find that the calculated time-averaged calcium is ~ 600 nM in myopathic vs. ~ 400 nM in isolated myocytes from non-failing hearts. This calculation shows that there is no shortage of $[Ca^{2+}]_i$ in myopathic hearts.

Similarly, the data can be viewed from the force production end. Total calcium reported (resting plus peak plus one standard deviation) for isolated human myocytes reported by Beuckelmann et al. [15] would be 1138 nM for control and 702 nM for myopathic. If *in vivo* force-calcium relationships reflect more closely the intact steady-state force calcium relationship, the difference in contractile activation would be 12 percent. In tetanized preparations, the $[Ca^{2+}]_{50\%}$ for control and myopathic human myocardium is 0.56 ± 0.05 vs. 0.54 ± 0.09 μM for control and myopathic, respectively, with Hill

coefficients of 5.21 ± 0.2 vs. 5.61 ± 0.6 [4, 7]. For skinned fiber preparations, the $[Ca^{2+}]_{50\%}$ and Hill coefficients are 1.56 ± 0.22 vs. $1.44 \pm 0.16 \mu M$, and 2.05 ± 0.33 vs. 2.3 ± 0.23 , respectively [4, 7]. Further, for twitch force, the $[Ca^{2+}]_{50\%}$ are 1 ± 0.02 vs. $0.72 \pm 0.13 \mu M$ with Hill coefficients 5.2 ± 0.08 vs. 4.57 ± 0.21 for control and myopathic myocardium, respectively [4, 7]. In this case, the difference between control and myopathic force is 8 percent. Such a relationship between force and calcium would be particularly applicable in failing human myocardium where the “apparent” force-calcium relationship is leftward shifted [4]. Failing human myocardium has a slower cross-bridge cycling rate [10] and differential effect of agents targeted at the level of the myofilaments [4, 5, 7]. Recently, it has been demonstrated not only that failing human myocardium undergoes changes in troponin T isoform content [41] but also troponin T isoforms affect calcium sensitivity and cooperativity as well.

Recent results gathered in multicellular trabeculae carnae from failing human hearts support this method of analysis of the relationship between peak force and calcium concentrations while raising new questions. Figure 12 shows the $[Ca^{2+}]_i$ and force responses of a left ventricular trabeculae carnae taken from a failing heart at 1.0 Hz and 1.5 Hz electrical stimulation rates at 32°C. With the increased stimulation rate, the systolic force decreased. The $[Ca^{2+}]_i$ response to an increased stimulation rate from 1 Hz to 1.5 Hz demonstrated significant differences. The peak $[Ca^{2+}]$ level is diminished by more than 50 percent suggesting that the intracellular calcium level has dramatically been reduced. However, in response to the frequency perturbation, the resting intracellular calcium concentration increased. Also similar to past findings, the configuration of the calcium

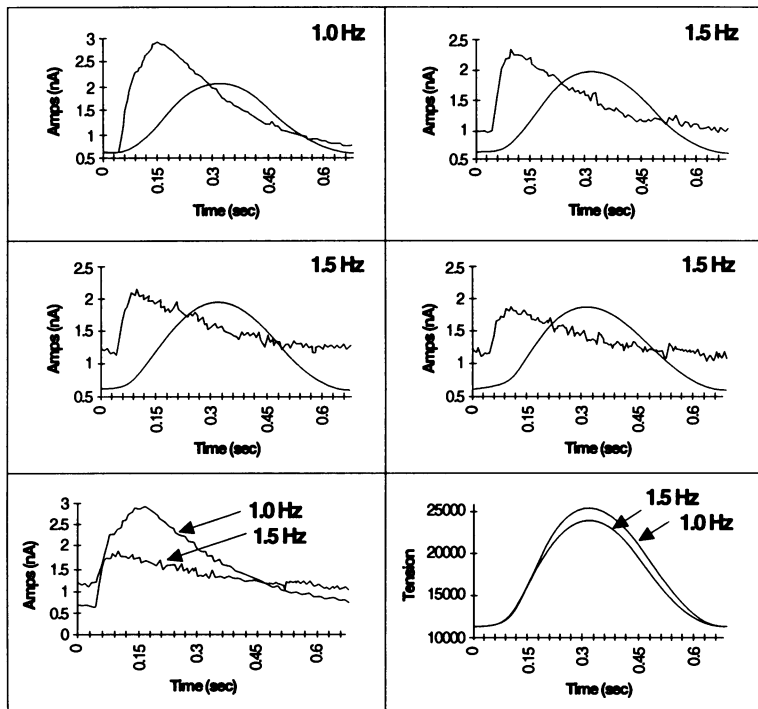


Figure 12. Calcium signals and isometric twitch at steady-state (1 Hz) and during transition to a new steady-state at a higher stimulation rate (1.5 Hz). Notice the decrease in peak amplitude of the calcium transient (noisy traces) reflecting predominantly SR calcium release as detected with aequorin (nA). Also note the increase in diastolic calcium concentrations as the frequency of stimulation is increased. Bottom panels are calcium transients and twitch force (smooth traces) superimposed at steady-state for 1 Hz and 1.5 Hz.

transient varied. The time-to-peak was shortened, while the time to 80 percent relaxation was prolonged. By reviewing the transient, not as peaks or levels, but rather as an amount, the data show that the relative amounts of $[Ca^{2+}]_i$ only decreased by 17 percent, a mere fraction of the noticeable 50 percent decrease in the peak of the calcium transient resulting in a 20 percent decrease in the systolic twitch force. Taking each variation in force and calcium into consideration, the data show that the force-time integral of the muscle only decreased by seven percent.

ENERGY SUPPLY AND DEMAND

Myocardial enzyme activity analyzes have shown significant differences between patients with angina (i.e., decreased lactate dehydrogenase activity) and those with normal coronary arteriograms [42]. Nascimben et al. have reported that creatine kinase activity, total creatine (creatine plus phosphocreatine) is significantly lower in ventricles from failing human hearts when compared to non-failing control hearts [11]. In addition, there is a higher content of MB creatine kinase that most likely reflects a response of the heart to increased wall stress. Changes in creatine kinase might result in diminished energy supply to ATPases and ionic pumps as well as the myofilaments (e.g., myofibrillar and myosin ATPase). Mitochondrial deletions have been reported in patients with idiopathic dilated cardiomyopathy as well as myocardial enzyme changes in alcoholic cardiomyopathy [43, 44]. Therefore, a mismatch between energy supply and demand could result at higher heart rates and increased workloads (e.g., increased wall stress and pharmacologically induced increased inotrophy, as is often seen in heart failure). The combinations of decreased energy reserves and abnormal calcium metabolism would contribute to the systolic dysfunction seen in patients with heart failure.

CONCLUSIONS

It is important to note that our earlier experimental findings [13] have been confirmed by others now studying human myocardium: i.e., similar levels of contractile force at slower stimulation rates [18-21]; prolonged action potentials [23, 28]; diminished responsiveness to phosphodiesterase inhibitors and cyclic AMP concentrations [19]; negative force interval relationship [21]; similar myofilament force-calcium relationships and ability to generate similar levels of force [6, 18-21]; and elevated resting calcium concentrations [15, 22]. Discrepancies appear to arise as one becomes further removed from intact functional physiology and, thereby, raises questions as to whether experiments done in test tubes or under "standard" conditions truly reflect intact muscle physiology. We therefore propose that although investigators assume they are studying similar disease states under similar conditions this may not be the case. Scientists in varying laboratories must be willing to experimentally repeat protocols so as to verify or refute reports in the literature. In the case of heart failure, the quest for information is too important for experimenters to seek novelty with regard to experimental protocol and experimental findings. For it may well be the novelty in experimental protocols that muddles the scientific literature with regard to scientific findings and, more importantly, data interpretation of the pathophysiology and pathogenesis of heart disease. A sharing of human heart samples as well as a united effort must be made by scientists using multiple techniques and animal models if the area of scientific investigation into the potential treatment of heart failure is to move forward.

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